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Methacrylate monolithic stationary phases for gradient elution separations in microfluidic devices

Peter Pruim[a], Marcus Öhman[b], Peter J. Schoenmakers[a], Wim Th. Kok[a,*]

[a] Analytical Chemistry Group, Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, P.O. Box 94157, 1090 GD, Amsterdam, The Netherlands
[b] Department of Chemistry, Karlstad University, SE-651 88, Karlstad, Sweden

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ABSTRACT

Methacrylate monolithic stationary phases were produced in fused-silica chips by UV initiation. Poly(butyl methacrylate-*co*-ethylene dimethacrylate) (BMA) and poly(lauryl methacrylate-*co*-ethylene dimethacrylate) (LMA) monoliths containing 30, 35 and 40% monomers were evaluated for the separation of peptides under gradient conditions. The peak capacity was used as an objective tool for the evaluation of the separation performance. LMA monoliths of the highest density gave the highest peak capacities (≈40) in gradients of 15 min and all LMA monoliths gave higher peak capacities than the BMA monoliths with the same percentage of monomers. Increasing the gradient duration to 30 min did not increase the peak capacity significantly. However, running fast (5 min) gradients provides moderate peak capacities (≈20) in a short time. Due to the system dead volume of 1 μL and the low bed volume of the chip, early eluting peptides migrated over a significant part of the column during the dwell time under isocratic conditions. It was shown that this could explain an increased band broadening on the monolithic stationary phase materials used. The effect is stronger with BMA monoliths, which partly explains the inferior performance of this material with respect to peak capacity. The configuration of the connections on the chip appeared to be critical when fast analyses were performed at pressures above 20 bar.

1. Introduction

Downscaling of liquid chromatography (LC) columns has progressed over the years from capillary LC to Nano LC and recently to the format of microfluidic devices. One of the important driving forces for these developments is the improved efficiency of electrospray ionization (ESI) at lower flow rates because of the smaller amount of liquid that needs to be evaporated [1]. A more efficient ESI process results in more sample ions that can be analyzed by the mass spectrometer, leading to more accurate compound identification and quantification. In the case of proteomics this means that peptides can be identified at lower quantities, resulting in more comprehensive proteome characterization.

Micromachining techniques can be used to manufacture microfluidic devices incorporating injection loops, topographic structured channels, mixers, electrospray nozzles and many more features [2–7]. So, where previously only the chromatographic column was scaled down to a smaller volume, micro technology currently offers the possibility to miniaturize the whole analytical system. The possibility to integrate various components on a single analytical platform gives almost unlimited opportunities for multiplexing, multidimensional separations and single molecule detection.

From a chromatographic point of view, arrays of pillars in a microfluidic channel may be regarded as an ideal stationary phase [8]. It has been shown that with such channels extremely low plate heights can be obtained in practice [9]. A drawback of the pillar arrays is the low surface area, which makes it difficult to perform interaction chromatography. However, advances are being made towards the production of topographic structures modified with a porous layer to increase the available surface area [10]. Still, there is a long way to go before this approach towards chip-LC will become widespread.

A more straightforward approach for the manufacturing of microfluidic separation devices is by packing a particulate stationary phase in a channel, just as in a conventional column. Various types of stationary phase particles have been packed in microfluidic devices (see e.g. [11], and references therein). Modified silica particles can be packed in channels in polyimide chips that can be used in various modes of chromatography [12,13]. This approach has been made commercially available by Agilent. A convenient alternative for packing of channels with particles is the use of monolithic stationary phase materials in chips. Monoliths can be produced in situ and covalently linked to the channel wall [14–17]. Different polymerization chemistries can be used to produce monolithic materials, but acrylate and methacrylate materials are most

* Corresponding author. Tel.: +31 20 5256539; fax: +31 20 5255604.
E-mail address: W.Th.Kok@uva.nl (W.Th. Kok).

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common. Acrylate- and methacrylate-based stationary phases are produced by means of a single-step polymerization reaction which can be initiated thermally or by UV light. The use of UV-initiation would offer an additional advantage: the possibility to precisely determine the position of the retentive phase in the microfluidic device. Moreover, methacrylate monoliths possess a high permeability allowing higher flow rates or low back-pressures, which may be important for devices with pressure limitations.

Methacrylate monolithic stationary phases have been introduced by Svec and Frechet over a decade ago [18] and since that time much effort has been put into the study and development of these materials. This has resulted in a wide range of available materials with different monomers incorporated in the polymer to perform various modes of liquid chromatography [19–25] and in an increased understanding of the factors determining the analytical performance [26–30]. In a fused-silica chip pressure-driven reversed phase separations of peptides and proteins using a stearyl acrylate monolith have been performed in the isocratic mode by Reichmuth et al. [31]. Le Gac et al. [16] performed gradient elution separations of a tryptic digest of Cytochrome C using a lauryl methacrylate stationary phase in a SU-8 based microfluidic device. Levkin et al. have shown separations of peptides and proteins in a chipLC system on different (thermally initiated) organic monolithic stationary phases [17]. In general, however, the performance of systems in chip format is still not matching that of column or capillary formats with packed or monolithic stationary phases [32].

In this paper we describe the production of methacrylate monolithic stationary phases in a channel on a fused-silica chip. The final goal of this study is to explore the possibilities of using such channels as part of a two-dimensional separation system on a chip. For the coupling of two different separation mechanisms the chip format will offer much more possibilities than a column or capillary format. Different reaction mixtures were tested to produce stationary phases that were compared with respect to their chromatographic performance in gradient reversed phase separations of peptide mixtures, and their permeability. Also some practical difficulties were addressed.

2. Experimental

2.1. Materials and reagents

The peptides leutinizing hormone releasing hormone (LHRH, 1), angiotensin 2 (2), [val^5]-angiotensin 1 (3), substance P (4), renin substrate (5), momany peptide (6), insulin chain B oxidized (7), and melittin (8) were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). Numbers between brackets correspond to the numbers used in the chromatograms to identify the peaks. The ingredients of the polymerization mixture, lauryl-methacrylate (99%, LMA), butyl-methacrylate (99%, BMA), ethylene dimethacrylate (98%, EDMA), 1,4-butanediol (99%) and azobisisobutyronitrile (98% AIBN), were also obtained from Sigma–Aldrich. Other chemicals were obtained from standard suppliers and used as received.

2.2. Microchip fabrication

The device consisted of two fused-silica wafers, which were assembled by direct bonding. Before processing, the wafers were cleaned by oxygen plasma and subsequently a wet cleaning with nitric acid. In the bottom wafer the 38 μm deep channel was etched with 25% HF, with a chromium/gold layer as mask material. The 30 nm chromium layer and 120 nm gold layer were deposited by sputter deposition and patterned by photolithography and wet etching. In the top wafer the entrance holes were powder blasted using a Microblaster MB1002 with 29 micron aluminum oxide powder. The mask material in this case was the photosensitive powder blast foil BF410, patterned by photolithography. After removing the mask material of both wafers and subsequent cleaning, the wafers were aligned and pre-bonded. To fix the bond, the wafer stack was subjected to a temperature treatment at 1100 °C for 1 h and slowly cooled down with a rate of 25 degrees per hour. Finally, the wafer stack was diced into chips with dimensions suitable for the Micronit Lab-on-a-chip kit 4515 (Micronit Microfluidics BV, Enschede, The Netherlands). The channel has a length of 40 mm in a chip of 45 mm × 15 mm. Fig. 1 shows a schematic representation of the cross section of the channel and its dimensions.

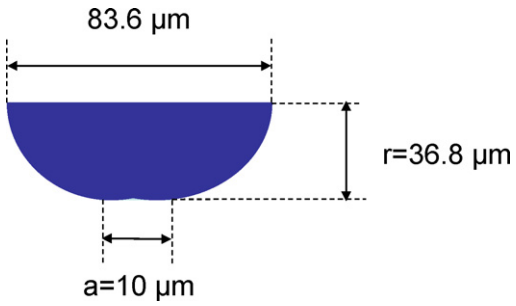

Fig. 1. Schematic representation of the cross section of the channel.

2.3. Preparation of monolithic stationary phases

Poly(butyl methacrylate-*co*-ethylene dimethacrylate) (BMA) and poly(lauryl methacrylate-*co*-ethylene dimethacrylate) (LMA) monolithic stationary phases were prepared in fused-silica chips by a single step UV-initiated polymerization reaction. The channel inner wall was vinylized before introduction of the polymerization mixture to ensure anchoring of the stationary phase [33]. The polymerization mixture consisted of BMA or LMA as a bulk monomer and EDMA as crosslinker in a 3:2 (v/v) ratio. Mixtures with 30, 35 and 40% monomers were evaluated. 1-Propanol and 1,4-butanediol were added as porogens in a 4:3 (v/v) ratio. The UV sensitive initiator AIBN was added in amounts of 1% (w/v) with respect to the monomers [34]. The polymerization mixture was sonicated and degassed before use. After injecting the mixture into the microfluidic device, the ends of the connecting capillaries were sealed with pieces of septum. A UV-Crosslinker (Spectroline, Westbury, NY, USA) was used to irradiate the exposed surface for 50 min at 254 nm with an intensity of 3 mW/cm^2. Before use, the monolith-filled chips were flushed extensively with acetonitrile (ACN) until a stabile back pressure was reached. After that, two fast gradient runs from 0 to 100% ACN were executed in order to remove any remaining porogens and possible particulates that would otherwise have negative effects on the performance of the MS. Then, the chip was wiped clean and interfaced with the MS. A limited number of chips were available and the method of Throckmorton et al. [35] was used therefore to re-use the chips. Chips of which the evaluation was completed, were heated overnight in an oven at 250 °C. After cooling the chips were flushed with a 1 M sodium hydroxide solution to dissolve and remove the – now incinerated – monolith.

2.4. Instrumentation and chromatographic conditions

Reversed phase separations were performed in the gradient mode using an Agilent 1100 series NanoLC system interfaced to an ion-trap mass spectrometer via an Orthogonal Nanospray ion source (Agilent Technologies, Waldbronn, Germany) with a picotip emitter needle of 5 cm length and 8 μm i.d. (New Objective, Woburn, MA, USA). Electrospray ionization was performed at